REMARKS

I. SUMMARY OF THE CLAIMED INVENTION

Claims 2-8, 10-14, 21 and 23-27 are pending in the application.

The present invention, as defined by independent claim 21, is a method for determining the presence of a phosphatase-targeting toxin in a sample comprising the steps of:

(A) contacting a first ligand, wherein said first ligand is immobilized on a solid support, with: (i) a sample suspected to contain a phosphatase-targeting toxin, and (ii) a second ligand,

wherein said first ligand is a protein phosphatase enzyme and is capable of binding, in a competitive manner, said phosphatase-targeting toxin and said second ligand;

(B) separating a bound fraction from a non-bound fraction, and

(C) determining the presence of said second ligand in the bound fraction, wherein the amount of said second ligand in the bound fraction is indicative of the amount of said phosphatase targeting toxin in said sample.

The concept of the present invention is illustrated in Figure 1 of the specification.

As described in the specification at pages 1-3, phosphatase-targeting toxins are produced by microalgae, such as cyanobacteria and dinoflaggellates, and present a significant problem, not only to the health of the consumer, but also to the shellfish industry, which must somehow monitor their products for contamination. As stated by Boland et al., *Toxicon* (1993), at page 1394 (of record):

For example, An & Carmichael, *Toxicon* 32:1495-1507 (1997) (a copy of which accompanies the Information Disclosure Statement submitted herewith) illustrates that antibody assays do not necessarily distinguish between non-toxic and toxic entities in the sample. Specifically, Table 1 of An & Carmichael shows several "non-toxic" entities (column 2) that gave a positive ELISA reaction (column 3). (See also Table 3 of An & Carmichael)

The limitations of immunoassays are further described by Boland et al., namely, that classical techniques such as immunoassays are not sufficient to analyze the toxicity of a sample due to, for example, the diversity of the toxins that may be present (see page 1394 of Boland).

On the other hand, the protein phosphatase enzyme inhibition assays suffer from non-specific inhibition of the enzyme, resulting in false positives, and are hampered by phosphatase contamination from the sample, which leads to an underestimate of toxin content and false negatives (as discussed by Ward et al. at page 472). To reduce these effects laborious fractionation /separation steps are required to remove such non-specific factors prior to the analysis.

In fact, in view of these limitations, An & Carmichael suggest at page 1505-1506 that both ELISA and phosphatase inhibition assays should be used to determine sample toxicity:

> As stated in the introduction, it is very important to have a sensitive method to monitor the quality of the drinking water sample for the presence of microcystin activity. In our study, a colorimetric PP1 inhibition assay was developed for this purpose. This colorimetric method was found to be very sensitive and able to detect the bioactive

> Up to 40 microcystin variants have been isolated to date as the products of water-blooms of cyanobacteria/blue-green algae such as *Microcystis, Anabaena, Osillatoria* and *Nostoc sp.* ***The increasing number of toxic variants being identified presents a formidable challenge to their detection by either immunoassay or classical instrumental analytical techniques in the absence of a sensitive biological assay*** (citations omitted, emphasis added).

To address these needs, the present invention provides a simple, sensitive, and inexpensive method for determining the presence of phosphatase-targeting toxins in samples suspected of containing these toxins.

The present invention addresses limitations in both the protein phosphatase enzyme inhibition assays and ELISA assays, which are commonly employed in the art. Specifically, the method of the present invention is less susceptible to interfering factors, when compared to protein phosphatase enzyme inhibition assays, and allows determination of the toxicity of the sample based upon the level of physiological toxins, as opposed to ELISA assays, which do not accurately reflect sample toxicity.

II. CLAIM REJECTION UNDER 35 U.S.C. §103(a)

At pages 3-6 of the Office Action, claims 2-8, 10-14, 21 and 23-27 are rejected under 35 USC §103(a) as being obvious over U.S. Patent 4,554,088 to Whitehead et al., in view of Ward et al., *FEMS Microbiology Letters* 153: 465-473 (1997).

Specifically, with respect to independent claim 21, the Examiner contends that Whitehead et al. teach an assay for isolation of molecules, the assay involving ligands attached to magnetic particles that may capture the complementary labeled or non-labeled ligand from a

sample. The bound complementary labeled or non-labeled ligand may then be separated from the unbound, and measured. The Examiner refers to column 7, lines 25-35, of Whitehead et al., which indicates that enzymes and their inhibitors are suitable ligands for the magnetic particle assay.

The Examiner acknowledges that Whitehead et al. fail to teach an assay for the presence of a phosphatase-targeting toxin, and which involves the use of a protein phosphatase as a ligand.

However, the Examiner contends that Ward et al. teach a colorimetric protein phosphatase inhibition assay for microcystins (a phosphatase-targeting toxin), and which uses a protein phosphatase enzyme. The Examiner contends that, according to Ward et al., microcystins bind irreversibly to, and inhibit, protein phosphatases 1 and 2A. The Examiner also refers to Ward et al. as teaching that, due to the increased awareness of the hazards presented by these toxins, increasingly sensitive detection methods are required to provide information for the effective management of waters supporting cyanobacterial blooms.

The Examiner concludes that it was *prima facie* obvious to one of ordinary skill in the art at the time the present invention was made to use the assay of Whitehead et al. to detect microcystins by using a protein phosphatase immobilized to magnetic particles.

III. APPLICANTS' RESPONSE TO THE SECTION 103(a) REJECTION

The rejection is respectfully traversed because the prior art does not provide any motivation to use an immobilized phosphatase as a ligand in a binding assay, and despite the significant effort in the prior art to develop an improved assay for determining sample toxicity,

the art does not teach the claimed method, which addresses several limitations of the conventional techniques.

(A) THE PRIOR ART DOES NOT SUGGEST THE USE OF AN IMMOBILIZED PHOSPHATASE AS A LIGAND IN A BINDING ASSAY

There is insufficient motivation to combine the teachings of Whitehead et al. and Ward et al., so as to render the present invention *prima facie* obvious.

Ward et al. (published in 1997) was published well after Whitehead et al. (issued 1985), and acknowledges the need for a more specific, sensitive, and convenient assay for microcystins (see page 466, left column). However, despite being armed with the knowledge of Whitehead, and despite the fact that techniques for performing binding assays such as that of Whitehead were well-known and commercially available well before 1997, Ward does not teach or suggest the use of a binding assay as presently claimed. Instead, the entire disclosure of Ward is directed to developing a colorimetric phosphatase inhibition assay (an enzymatic assay).

Because Ward was faced with the same problem as that of the present inventors, and because Ward chose a different route in an attempt to solve that problem, Ward does not motivate one of ordinary skill in the art to use an immobilized phosphatase as a ligand in a binding assay as claimed. In fact, Ward teaches away from the present invention by guiding one of ordinary skill in the art to colorimetric enzymatic assays.

Thus, the prior art does not provide any motivation to determine the presence of phosphatase-targeting toxins in samples using a binding assay that employs an immobilized phosphatase as a ligand. This becomes especially evident in view of the long-felt need for an

improved assay for detecting phosphatase targeting toxins (discussed below), and the clear and unexpected advantages of the present invention (also discussed below).

(B) THE PRESENT INVENTION ADDRESSES A LONG-FELT NEED IN THE ART

The art already of record in this Application shows that there was a "long-felt need" for a sensitive yet reliable assay for detecting phosphatase-targeting toxins. The Examiner is requested to consider the following teachings and suggestions in the art, which must be considered in the obviousness determination.

Usagawa et al., *Toxicon (1989)* states at page 1323:

> Diarrhetic shellfish poisoning was first reported in 1979 at the Tohoku district in Japan by Yasumoto et al. (1980), and since then has been shown to be prevalent worldwide. ***In recent years concerns have been expressed from the viewpoint of safety of shellfish products and deleterious effect on the shellfish industries.*** (emphasis added)

U.S. Patent 5,180,665 to Holmes, filed November 21, ***1990***, states at column 2, lines 42-44:

> However, when crude marine extracts from shellfish or phytoplankton are assayed for okadaic acid using a protein phosphatase, it is difficult to evaluate whether the inhibitory response results from heterogeneous or homogeneous activity. Hence, since okadaic acid is not the only compound which inhibits the enzymatic activity of protein phosphatases, and this may be especially important in the case of shellfish which are filtering organisms, ***it is virtually impossible to determine the organisms, presence of okadaic acid simply by analyzing the inhibitory effect of a crude sample on a given protein phosphatase. Thus, there is still a great***

> ***need for an efficient marine bioscreen for DSP toxins such as okadaic acid and related derivatives***. (emphasis added)

U.S. Patent 5,264,556 to Sikorska, filed March 27, ***1992***, states at column 2, lines 24-26:

> The assay [of UBE Industries of Japan] is very expensive due to the use of okadaic acid as a capture antigen. Okadaic acid is extracted from sponges, collection and processing of which is very expensive. The kit in the present form is not marketable for general, prophylactic testing of seafood and plankton by fish industries due to its high cost and complicated sample preparation. ***It would be highly desirable to have an assay for the measurement of okadaic acid which would be accurate, sensitive, easy to use and inexpensive***. (of record)

U.S. Patent 5,525,476 to Matsuura et al., filed August 10, ***1992***, state at lines 12-24:

> The diarrheal shellfish poison is the second most frequent type of food poisoning after blowfish in terms of number of outbreaks, but is number one in terms of number of victims, and ***therefore is a major problem in food sanitation.*** (emphasis added)
>
> Hitherto, the measurement of lethal activity using mice is adopted as the official method of examination of diarrheal shellfish poisons, but there were problems in terms of the management of the animals, the sensitivity of detection, the precision, and the specificity. ***On the other hand, attempts have been made to develop techniques aimed at performing the above examination with a high sensitivity, in a simple manner, and in a short time.*** (emphasis added)

Boland et al., *Toxicon* (***1993***), state at page 1394:

> Up to 40 microcystin variants have been isolated to date as the products of water-blooms of cyanobacteria/blue-green algae such as *Microcystis,*

> *Anabaena, Osillatoria* and *Nostoc sp.* ***The increasing number of toxic variants being identified presents a formidable challenge to their detection by either immunoassay or classical instrumental analytical techniques in the absence of a sensitive biological assay*** (citations omitted, emphasis added).

Simon et al., *Natural Toxins* (***1994***), state at page 293: "A cheap, simple, sensitive, and rapid method for the analysis of okadaic acid is needed for food control and biochemical investigations in the cell."

Honkanen et al., *Toxicon* (***1996***), state at page 1386:

> The original diagnosis of DSP was in 1976, in Honshu Japan. Since then, cases have been reported in Scandinavia, Spain, Mexico, The Netherlands, France, South America, Australia, and Canada, and phytoplankton-producing DSP toxins have been identified in temperate coastal waters worldwide. In addition, some evidence suggests that the number of toxin-producing phytoplankton may be increasing as a result of coastal eutrophication. ***Thus, the accurate identification of seafood which contains DSP-producing toxins may be useful for preventing human poisonings and should also aid in the efficient management of potentially contaminated shellfish***. (citations omitted, emphasis added)

Ward et al., *FEMS Microbiology Letters* (***1997***), state at page 466:

> Due to the increased awareness of the hazards presented by these toxins, and the recommendation of guideline values for potable waters, ***increasingly sensitive detection methods are required to provide information for the effective management of waters supporting cyanobacterial blooms***. Current methods for the detection and quantification of microcystins include high-performance liquid

chromatography (HPLC), small animal bioassay, immunocytotoxicity and enzyme-inhibition assays. ***These differ in their specificity, sensitivity, ease of use and ability to provide qualitative and/or quantitative information regarding the in vivo or in vitro toxicity of samples.*** (emphasis added)

Thus, while the prior art of record shows a significant effort towards the development of a sensitive and reliable assay for the presence of phosphatase-targeting toxins, the prior art does not teach or suggest the critical feature of the present invention, the use of a protein phosphatase as a ligand in a binding assay.

Especially when viewed in context with the clear advantages of the present invention over conventional techniques, as shown by the present specification and as discussed below, the present invention is clearly non-obvious.

(C) THE PRESENT INVENTION IS SUPERIOR TO BOTH PROTEIN PHOSPHATASE ENZYME INHIBITION ASSAYS AND ELISA ASSAYS

Apart from the impractical and costly instrumental analytical methods, and the unethical, non-reliable, imprecise animal bioassays, the conventional methods employed in the art to detect phosphatase-targeting toxins are: (1) the antibody-based assays and (2) the protein phosphatase inhibition-based assays (enzymatic assay).

Both of these methods have their limitations.

First, antibody assays do not accurately reflect sample toxicity, because antibodies will not recognize all toxin congeners of a certain toxin group, and more importantly, do not recognize molecules based upon their toxicity.

> microcystins used in the study. The major drawback of the protein phosphatase method is its possible reaction with nonspecific phosphatases in the sample or its reaction with endogenous protein phosphatases that will lead to an underestimate of the toxins. ***We believe that a combination of an immunoassay such as ELISA and the colorimetric PP1 inhibition assay will prove very useful in detecting many of the microcystins and nodularins in environmental samples***. (citations omitted, emphasis added)

Thus, to solve the problems associated with both assays, An & Carmichael suggest using both assays, to complement the deficiencies of each: the immunoassay would detect components in the sample capable of binding to the antibodies employed, whereas the enzymatic assay would be used to indicate the toxicity of the sample.

The present invention, however, provides a single assay that overcomes these deficiencies.

For example, the results shown in Example 6 and the Table on page 19 of the present specification, demonstrate that the claimed method is much less susceptible to exogenous inhibitory compounds than the enzymatic assay exemplified by Holmes and Ward. As can be seen from the Table on page 19, compounds such as ATP, NaPPi, NaF, caseine, and histone 2A, significantly affect the results of the protein phosphatase assay, while having essentially no effect on the competitive binding assay of the invention.

This finding, that a binding assay employing a protein phosphatase as a ligand is less susceptible to interfering factors, was completely unexpected and could not have been foreseen from the teachings of the prior art.

In addition, as shown in Example 5, the present invention detects numerous toxins. Specifically, Example 5 shows that, when using an immobilized protein phosphatase, the presence of nodularin, microcystin-LR, microcystin-YR, okadaic acid, calyculin A, and tautomycin could be detected. This is in stark contrast to antibody assays that typically detect only a particular toxin.

Further, because the protein phosphatase is a physiologically relevant ligand, the method of the present invention will exclude analysis of inactivated toxins.

Therefore, in view of these very clear advantages of the present invention, which are set forth in the specification, and in view of the effort in the art to develop such a versatile and sensitive assay for toxicity, the present invention is non-obvious.

Withdrawal of this rejection is requested.

(D) CONCLUSION

Reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited.

If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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